CARNEGIE INSTITUTION OF WASHINGTON

ANNUAL REPORT OF THE TORTUGAS LABORATORY

[Reprinted from Year Book No. 31, for the year 1931-32, pages 279 to 292.
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TORTUGAS LABORATORY[1]

W. H. Longley, Executive Officer

The Laboratory was open from June 1 to August 21, 1932, during which time the following investigators worked for the periods and upon the problems indicated.

Paul Bartsch. U. S. National Museum. Cerion hybridization. August 10 to 21.
A. A. Boyden. Rutgers University. Serological study of invertebrate relationships. June 1 to July 25.
L. R. Cary. Princeton University. Invertebrate tissue culture. July 27 to August 21.
M. W. de Laubenfels. Pasadena Junior College. Physiology and taxonomy of sponges. June 15 to August 8.
F. R. Hayes. Dalhousie University. Nitrogen in early echinoid ontogeny. June 1 to August 8.
James L. Leitch. University of California. Water exchange of cells. June 1 to July 11.
Warren R. Lewis. Johns Hopkins University. The effect of selected indophenol dyes on fishes. June 15 to July 25.
W. H. Longley. Goucher College. The habits and distribution of fishes. June 1 to August 21.
H. W. Manter. University of Nebraska. Taxonomy and life-histories of trematoda. June 15 to August 8.
Waldo L. Schmitt. U. S. National Museum. The bathymetric distribution of decapod crustacea. June 15 to August 8.
R. G. Stone. University of Missouri. The effect of radium radiation on regeneration in *Euratella*. June 1 to August 8.
John W. Wells. Cornell University. Reef corals. June 15 to August 8.
O. L. Williams. University of California. Nematode parasites of fishes. June 1 to August 8.
Shigeo Yamanouchi. University of Chicago. Life-histories of algæ. June 1 to July 11.

During the season authorized repairs were made upon laboratory buildings. Earlier the launch *Velella* was rebuilt as contemplated, again giving the Laboratory two serviceable boats of intermediate size for routine work of collecting or for transferring working parties from key to key within the group. The addition of 500 fathoms to the cable used in dredging from the *Anton Dohrn* makes it possible to operate in 600 fathoms, which is as great depth as she may explore while retaining Tortugas as her base.

For details concerning the scientific work of the season, the reader is referred to investigators' individual reports, to which the following may serve as an introduction.

Dr. Bartsch has increased his plantings of cerions in mixed colonies with the idea of facilitating their crossing and increasing the material available for studying its effects. The work is of especial value in connection with his own studies of the local distribution and evolution of terrestrial mollusca of Cuba, other West Indian islands and the Philippines.

[1] Located at Tortugas, Florida.

Accepting the fact of evolution, and without immediate interest in its mode, Dr. Boyden attempts by serological methods to determine the closeness of the relationship between some of the invertebrate types to which it has given rise. Some of the species with which he is working are of uncertain affinity and his findings may be awaited with interest.

The researches of Doctors Cary, de Laubenfels, Hayes, Leitch and Stone and Mr. Lewis directly or indirectly deal with problems of growth and differentiation.

Dr. Cary has so far been largely and successfully engaged in developing methods of tissue culture readily adapted to use in the ordinary marine laboratory. It is to be hoped he may next apply them in study of problems presented attractively by marine invertebrates.

Dr. Stone, who by courtesy of the National Research Council's Committee on Radiation has enjoyed the use of radium, has brought to successful conclusion a study continued through two seasons. He finds that the beta rays are chiefly responsible for the diminished powers of regeneration displayed by the tissues of irradiated worms in his experimental cultures.

Mr. Lewis's attempt to discover the effect upon pigmented dermal cells of fishes of certain dyes known to decolorize similar cells in living tadpoles has perhaps failed for the moment, at least, on account of their failure to be absorbed. A modification of procedure may perhaps eventually open the way for profitable investigation of the pigmented, cancerous growths of fishes from this new direction.

Dr. Manter's study of the trematode fauna of Tortugas, in collection of material for which he has enjoyed the assistance of Dr. O. L. Williams during the season, has progressed notably. His data promise to have much value not only as a contribution to systematic knowledge of a group of animals with respect to which much remains to be learned, but to lend themselves to use in a connection mentioned in last year's report. This phase of his research dealing with geographical distribution is part of a joint investigation in which Dr. Schmitt and Dr. Longley participate.

Cerion Breeding, by Paul Bartsch

This year's efforts were centered about two problems: First, an examination of the existing colonies at the Tortugas, particularly the status of that of the first generation of hybrids between *Cerion incanum* and *Cerion viaregis* reported upon last year. Only five of the eight have survived. Secondly, the establishing of new mixed colonies of *Cerion incanum* and *C. viaregis;* and *Cerion incanum* and *C. casablancæ.* Two such colonies, consisting of 500 of each species, were planted.

One of these, *Cerion incanum* and *C. viaregis,* is placed on the south end of Loggerhead Key, the place being marked by a stake bearing a metal plate with proper legend. Another mixed colony of the same combination has been planted on the southwest corner of Garden Key outside of the Fort.

Of *Cerion incanum* and *C. casablancæ* a mixed colony of 500 each was planted near the pump house on the north side of the laboratory on Loggerhead Key, and another of an equal number on the northwest elevated reach of Long Key.

From these new colonies it is hoped to get sufficient first generation hybrids from which we may obtain the second generation of progeny.

The second combination was made desirable because we found this crossing to have taken place on Bahia Honda Key last year. The status of the free colonies living on the various keys is satisfactory.

The Bird Rookeries of the Tortugas

The usual breeding colonies of sooties, noddies, common, roseate and least terns were present in the quarters previously occupied, but several decided changes in these tern colonies are to be recorded, namely, that about twenty pairs of the least terns were breeding on the hook at the north end of Loggerhead Key this year. By far the most interesting development in these tern colonies centers this year about Bird Key, which has again suffered a decided diminution from wind and weather with the result that it scarcely offers adequate quarters for the large number of birds that it has been harboring, and this has resulted in the first attempt at an exodus so far recorded.

About 100 nests, probably 70 of the noddy and 30 of the sooty, were found on the elevated portion of Bush Key in an exceedingly retarded condition of development, some still containing eggs, others newly hatched young and the most highly advanced being less than half-grown at the end of our laboratory season, while on Bird Key almost all the birds were on wing. The noddies of this colony occupied the pads of *Sesuvium,* and the sooties, as usual, the intermediate stretches of sand. This is a very unfortunate selection for the first real stormy sea is sure to wash over the place. A better selection is that of another group of noddies, who have made about 32 nests in the few tall bay cedars at the western end of Long Key.

A third unfortunate choice was made by another small group of noddies who selected the tops of groups of massed piles on the south side of the east dock of Fort Jefferson on Garden Key. The young raised here when old enough to move about, some half-grown, had evidently fallen overboard and been lost.

This restlessness on the part of the Bird Key tern colony is more than interesting, and it should be remembered that the sooties and noddies breed nowhere else in the United States. The logical key that should be selected by them is Loggerhead Key whose bay cedars and sandy reaches would furnish an ideal habitat for these birds, but here they would prove a decided nuisance to both our laboratory and lighthouse people, both of which are dependent upon rain water and cisterns for their water supply, which the droppings of these birds would render impossible.

Photographs

Time and weather permitting, 1300 feet of moving picture film were exposed: 300 showing the present status of the bird rookeries on Bird Key and the new sites chosen by the terns, and 1000 feet undersea about the Coral Reef.

Serological Study of the Relationships of some Common Invertebrata, by Alan Boyden

The precipitin reaction, known to possess certain advantages over other methods of studying systematic relationships, has heretofore been applied chiefly to the investigation of relationships within the Vertebrata. The heterogeneous assemblage of Invertebrata, whose interrelationships are much less certain, has scarcely been touched. The collection of materials to serve as antigens in such an investigation was begun at Tortugas.

The method of antigen preparation includes the following steps: (1) Collecting and starving the animals; (2) obtaining the body fluids (where present) and grinding the tissues with the addition of sterile salt solution; (3) vigorously shaking the ground tissues in a shaking machine; (4) filtering the extracts, ending with a final sterilizing filtration through Seitz filters; (5) storing in sterile condition in 5 ml. serum vials; and (6) testing for sterility and protein concentrations.

In some cases the extracts were concentrated by blowing with electric fans before the final filtration.

What appear to be satisfactory antigens for use in the production of antisera, were obtained for 28 genera distributed as follows: Porifera, 2; Cœlenterata, 1; Sipunculoidea, 1; Annelida, 3; Crustacea, 5; Mollusca, 7; Echinodermata, 6; Prochordata, 3.

In addition, other antigens representing seven more genera were obtained which will be suitable for titration, even though not concentrated enough for injection. They are distributed as follows: Cœlenterata, 4; Mollusca, 1; Echinodermata, 1; Prochordata, 1.

Finally, samples of the blood sera of three of the lower Vertebrata were obtained, to be used in the study of Prochordate affinities.

The next step in the investigation will be the production of the precipitating antisera and this will be followed by the making of the actual titrations by means of which further light may be thrown on the systematic relationships of the species studied. The results should help to make more clear the proper location of those species of "uncertain systematic position," and to make more quantitative the expression of the interrelationships of all the forms studied.

Report on Tissue Culture, by L. R. Cary

My work during this season was confined to a study of the behavior *in vitro* of tissues of *Ptychodera bahamensis*. The routine procedure for making the cultures, described in my report of last year, gave uniform success.

Sterilization of tissue fragments by irradiation with ultra-violet from a mercury vapor-bulb was equally efficient. This procedure resulted in a marked saving of time. There was no apparent deleterious effect on the tissues from an exposure of 2½ minutes at 15 cm. All bacteria were destroyed or rendered incapable of reproduction for several days. After a week, bacterial activity sometimes became very marked in cultures prepared by this procedure. A peptic digest of entire Ptychoderas was used as a nutrient medium in the preparation of all cultures.

Last season almost complete failure resulted in attempts to obtain satisfactorily stained preparations from the cultures, as a permanent record of their activities. Consequently an extensive series of photomicrographs of cultures throughout the period of their development was made during the first two weeks of this season. Later a series of micro-cinematographs of the growth of explants from the digestive cæca were taken.

The structure of *Ptychodera* makes it possible to choose as an explant a small fragment of tissue from the dorsal side of the mid-section of the body which will contain: (1) The ectodermal epithelium; (2) nerve cell; (3) cells from the digestive (hepatic) cæca; (4) muscle cells; and (5) connective tissue cells. In a culture from such an explant the first visible growth is that of nerve fibers. Then endoderm cells from the cæcum begin to migrate as flask-shaped bodies. Their stalks become very long and slender. Before these primary migrants have separated from the explant, other cells, to which the former are attached, move out from the mass of tissue. This process continues until often a chain of ten or more endoderm cells extends out from the explant. In the meantime many muscle cells become separated from the tissue mass. Their behavior varies greatly. Some become greatly elongated and show marked amœboid activity. Others shorten to masses of rectilinear shape. These soon swell until a clear vacuole surrounds the central mass of protoplasm. Later these may elongate and become extremely active.

By the time the greatest activity of the above-mentioned cells has been passed, a mass migration of the ectodermal epithelial cells takes place. This has the appearance simply of a flowing of the cell mass. Soon the other types of cells are entirely overgrown and hidden by the migrating ectoderm cells. The culture now appears as a smooth mass of rounded cells and all evidence of earlier activity of other elements is lost.

When an explant is selected which consists of cæcal endoderm alone, migration continues until no central mass is distinguishable. The component cells of the culture move about actively over the cover-slip. The protoplasmic strands between cells become highly attenuated. Relatively large areas are seen where only these connecting strands are to be found.

At the time of the division of the cells, their characteristic pigment granules may be passed on entirely to one daughter cell. The other now appears as a clear mass of protoplasm. When freed in this manner from the large granules, the cells exhibit unusual amœboid activity. They lose their characteristic shape and can not easily be distinguished as descendants of their parent cells.

Subcultures were easily obtained from many slides. When a fragment of the original explant was used, activity was always very marked. Indeed, subcultures from slides on which there had been no growth were usually very active as though the explant had been released from some inhibiting influence operating in the original culture.

Subcultures containing pure cæcal endoderm cells were easily obtained and kept alive for a period of two weeks without transfer. When transfers were made every 48 hours, these cultures could be carried on indefinitely.

Studies Upon Tortugas Sponges, by M. W. de Laubenfels

In continuation of my work on sponges at Tortugas, particularly with reference to methods of regeneration, cylindrical species were cut transversely and kept under observation while the injury was repaired. Sometimes disks were cut from such sponges, kept between glass, and watched while the new surface was formed, in this case not across the wound but out to the glass. Active moving about of cells in and from the sponge was involved, and some metamorphosis of one cell sort into another.

The commercial sponge *Spongia officinalis* (Linné) was more abundant this year than earlier, but was unsuited for experimental work with dissociated cells, because its cells refused to come out of suspension even when centrifuged.

A few observations were made upon sponges eaten by fishes under normal conditions, and upon the effect of feeding suspensions of sponge cells to others.

The specific descriptions of sponges by early students of the West Indian fauna are often so brief as to be worthless. There are excellent recent papers, but the number of species of which they treat is not large, and

very real need for additional systematic study of the group still exists. It seemed good, therefore, to undertake it now, since the season's dredging for other purposes yielded incidentally an abundance of sponges from depths ranging from 15 to more than 1000 m.

The dredged specimens were often studied while still alive and usually before post morten changes had set in. There numbers were supplemented by material collected with sponge-hook or diving-hood, or by hand from the reef at low tide. Over 80 species in all were obtained. Examples of each, preserved in alcohol, are being deposited in the U. S. National Museum, accompanied in many instances by representative series of dry specimens. Microscopic preparations of each were also made, with descriptive notes, and notes upon the ecology of the species where possible.

Nitrogen in Echinoid Ontogeny, by Frederick Ronald Hayes

After the penetration of a spermatozoon, the developing egg receives nothing from the outside except water and sometimes salts, until the comparatively advanced embryo begins to eat. The morphological phenomena of ontogeny—intra-cellular reorganization, cell division, gross changes in size and shape—can be brought about only by the expenditure of energy, and this energy must come from materials in the egg at the time of fertilization. The problems of chemical embryology include (*a*) a determination of the amount of energy required to produce these structural changes—the overhead expenses of development; and (*b*) an investigation of the chemical transformations taking place. Using sea-urchin eggs as material, the former problem received attention some years ago from Warburg and others. Oxygen requirements, carbon dioxide output, and heat production at various stages, suggested that the same material was not being burned to provide energy at all times. Virtually nothing has been done, however, which throws light on the chemical changes during invertebrate egg development. This therefore seemed a suitable field for a preliminary investigation at Tortugas, with the sea-urchin (*Echinometra lucunter*) as material.

There are two sources of energy available in the egg—proteins and lipins. (Probably the very small quantities of carbohydrate present can be neglected.) In the time available, an attempt to make a general survey of the changes in these two classes of material would necessarily have been unsatisfactory. It was therefore decided so to limit the scope of the work that a clear-cut result might be anticipated.

Primary amino groups were found to account for nearly 40 per cent of the nitrogen in *Echinometra* eggs. Now $-NH_2$ nitrogen is known to change in many metabolic processes, particularly with respect to its relation to $=NH$ and $\equiv N$ nitrogen. A study of the variation in the ratio $\frac{-NH_2 \text{ nitrogen}}{\text{total nitrogen}}$ during the first 24 hours of development was made, for the purpose of gaining some idea whether profound protein transformations accompany cleavage, hatching and gastrulation. Koch's modification of van Slyke's micro-apparatus was used for the estimation of $-NH_2$ groups; and micro Kjeldahl tests mere made for total nitrogen. The results showed that, although there may be small variations, no major change occurs in the ratio investigated. From this it may be suspected that there is probably little change in the protein during the early stages, although a quantitative estimation of the several amino acids present would be necessary before a definite conclusion could be reached. It might be further

reasoned that since chemical changes of some sort almost certainly form a part of development, it would be profitable to make a study of the lipins.

Some preliminary observations were made of the size changes during the first 24 hours. Measurements of diameters showed that the egg within the shell (or fertilization membrane) decreased in size until hatching time (6 to 7 hours), following which there was a period of rapid growth. The diameter of an egg is 85 to 90μ. Progress was made toward the elaboration of a method by which these small eggs may be weighed. Weighing is more desirable than diameter measurement for purposes of volume estimation, because with the latter method one must assume that eggs are spherical, which is not usually true.

Water Exchanges of Cells, by James L. Leitch

The object of the summer's work was to study the whole process of water exchange between ova of suitable types, particularly those of several echinoderms, and hypo- and hypertonic sea-water solutions. The program included the collection of samples for subsequent analysis and the measurement of the diameter of the eggs placed in anisotonic sea-water solutions. These measurements were made either by means of a filar ocular micrometer or by photographing the eggs and measuring the negatives. In all, some 320 photographs were taken which will be measured and the resulting data calculated during the coming year.

The ova of *Tripneustes esculentus, Lytechinus variegatus* and *Centrechinus antillarum* were not found in sufficient abundance or in proper condition for use.

The eggs of *Echinometra lucunter,* which was obtained in great number from Bird Key Reef, were used in the majority of the experiments. The eggs were obtained free from contamination by sperm or fluids from the cœlom or digestive tract by washing the animals in tap-water and then inverting them in a dish of sea-water. The animals spawned within 15 minutes. Photographic records of the volume changes when eggs were transferred from 100 per cent sea-water to 50 per cent and vice versa were made. From these photographs, studies will be made of: (1) The kinetics of the process of water exchanges; (2) the non-solvent volume of the eggs; (3) the effect on the non-solvent volume of keeping the eggs for 40 hours in the ice box; and (4) the variations in the kinetics of the volume changes for different samples of eggs from the same female and for samples of eggs from different females.

In addition to the photographic records, samples of eggs from each female used in the above experiments were thoroughly washed with filtered sea-water and prepared for analysis after the removal of most of the sea-water by centrifuging. Samples of the eggs of 40 females were also prepared in this manner for the determination of the variability in their chemical composition.

In preparation for future study of the reactions of eggs of animals of other phyla to hypo- and hypertonic sea-water solutions, a series of 56 pairs of samples of the eggs of individual female hermit crabs, *Calcinus tibicens,* were prepared. Each pair of samples consisted of a few eggs placed in Bouin's solution for a study of the shape of eggs at various stages in development, and a much larger sample for analytical study. The latter was prepared by washing the bunches of eggs in two changes of distilled water and then placing them in small vials in which they were dehydrated at 100° to 110° C. In the same manner, paired samples were made of the

eggs from one female of *Panulirus argus,* 4 of *Acanthocarpus alexandri,* 1 of *Stenocinops spinosissima,* 3 of *Portunus spinicarpus,* and 9 of *Mithrax verrucosus.*

Effects of Selected Indophenol Dyes on Fishes, by Warren Reed Lewis

Tadpoles which develop in solutions of certain of the phenol indophenol dyes lose the pigment from the skin and also from the eyes (M. R. Lewis). Therefore it was thought interesting to see whether these dyes could bring about a similar destruction of the pigment of other types of animals, particularly of the marine fishes.

Investigations were undertaken at the Tortugas Laboratory in collaboration with Mr. W. R. Kenny who prepared a number of dyes with these experiments in view. The dyes used were o-chlor phenol indophenol, phenol indophenol, both the Na salt and the free acid forms, and 1 naphthol 2 sulphonate indophenol which give a range of reduction potential from +0.233 to +0.123. The concentrations used were 1:100,000 and 1:200,000. Members from 4 families of fish were used: *Halichœres bivittatus* (Bloch), *Bathygobius soporator* (Cuvier and Valenciennes), *Malacoctenus moorei* (Evermann and Marsh), *Pomacentrus analis* (Poey).

No changes were observed in the living fish after four weeks, even though the concentrations of the dyes used in these experiments were much stronger than those used in the experiments on developing tadpoles.

The results seem to indicate that the dye did not penetrate the skin. However, in order to determine this, the preserved materials will be prepared in sections for microscopical observation, and in addition some of the sections will be oxidized to show whether any of the dyes may have passed through the epidermis in a reduced form.

A few types of invertebrates were tried but none survived under the conditions of the experiments.

Observations Upon Tortugas Fishes, by W. H. Longley

Chiefly as an incidental result of dredging undertaken for other purposes, several species have been added as usual to the local fish fauna as a result of the summer's work. There has not yet been opportunity to identify all precisely. The list includes at least these: *Antennarius* spp., *Chætodon aya, Cryptotomus roseus, Epinephelus niveatus, Gillellus* sp., *Gymnachirus fasciatus, Porichthys porissimus* and *Scorpæna inermis.*

Information has also been obtained upon a matter which attracted attention a year ago. Specimens of *Prionodes phœbe* and two other unnamed species of the genus, to the number of 40 in all, collected at different times and places, were without exception apparent females. Actually, it appears now, all three species are normally hermaphroditic. Active sperm cells in abundance have been taken from fishes of the two unnamed species whose eggs were at the same time almost ripe. Specimens of *P. phœbe* examined were not so nearly sexually mature and gave a less satisfactory demonstration of its bisexuality. It is to be anticipated, however, that the hermaphroditic condition prevails widely in the genus, for 11 mature specimens, distributed among four other species which there has been opportunity to examine, are also to superficial examination females only. None has a normal testis.

Actually the condition is by no means confined to the genus *Prionodes. Diplectrum bivittatum*—a species perfectly distinct from *D. radiale,* with which it has been confused—is also a functional hermaphrodite from which

I have had living sperm cells, together with eggs almost or quite mature. *Diplectrum formosum* is not mature at Tortugas at the end of August, but its immature gonads are all of one type, resembling immature ovaries. Four sexually mature museum specimens contained eggs, and one examined closely showed between the lobes of the gonad and behind the point of their union on the ventral side just such a mass of tissue as in *D. radiale,* and, in the species of *Prionodes* in life, yields sperm.

Hypoplectrus is another genus which, with little doubt, will prove to be like these in its mode of reproduction. I have seen 17 ovigerous museum specimens but no definitive males. Again the gonad of a specimen closely examined showed proximally on the ventral side a region resembling that from which the sperms of the other hermaphrodites come. In two of Poey's types in the Museum of Comparative Zoology this apparent spermatogenous tissue extends distinctly upon the lateral face of the gonad.

Dules auriga with 8 ovigerous specimens but none with normal testes promises to be another of the same sort. In any case, these observations with those of others show that normal hermaphroditism occurs very commonly among the lesser Serranidæ, though the conclusion does not apply to the genus *Pronotogrammus.*

A part of the summer was devoted to study of the bathymetric distribution of fishes within the 600-fathom line, but much the greater portion was spent in checking and organizing for publication data regarding species known from within the 100-fathom line. A note upon progress made will be found elsewhere in this volume.

Continued Studies on Trematodes of Tortugas, by H. W. Manter

Examination of fishes of Tortugas for helminth parasites has been continued another summer, this season with the assistance of Dr. O. L. Williams. Approximately 2400 fishes including 272 species have now been examined, almost all of them individually. Of the species of fishes, 80 per cent are subject to trematode infection. About 60 additional species of trematodes were added to the Tortugas list during the summer, bringing the total number to over 210 species from fishes alone. Of these, approximately 33 are monogenea. A large number of the fishes were secured by trawling at varying depths down to and including 582 fathoms. At all these depths the fishes were found to be more or less infected with trematodes and the indication is that the fairly deep ocean is well populated with these parasites.

Many new host records were secured. These, it is hoped, will contribute to correct understanding of specificity and distribution within this group of parasites. Knowledge of bathymetric distribution of certain trematodes is also growing. It is clear that there are rather definite vertical limits varying with the species. Most of the deeper-water forms belong to different species than those found near the surface, and enough collections have been made to give considerable significance to the upper and lower limits of occurrence.

Among the few species of trematodes of Tortugas also occurring north to Woods Hole is the form described from Beaufort by the author as *Rhagorchis odhneri* Manter 1931 (Parasitology, vol. 23, pp. 405-406). This species is, in reality, the same as the *Distomum pallens* Rud. of Linton 1898 (Proc. U. S. Nat. Mus., vol. 20, p. 526) and the correct name for the species depends on the confirmation of identity with Rudolphi's form.

The distribution of *Distomum fenestratum* at Tortugas is exceedingly wide. The parasite occurs, always in juvenile form, in many (at least

22) widely different hosts, including some from 168 fathoms as well as from shallow-water fishes. A massive infection in a ray (*Pteroplatea maclura*) from 60 fathoms shows that very heavy infections may occur in nature. There are probably at least two other species at Tortugas related to *Distomum fenestratum* but with much smaller ventral suckers. The Cercaria L of Miller 1925 (Carnegie Inst. Wash. Year Book No. 24, p. 238) from *Crepidula aculeata* seems to resemble this species of trematode very much since the large vesicles or swollen regions of the cœca are characteristic. It is possible that the sexually mature adults are to be looked for among the Didymozoonidæ.

The life cycle of *Helicometrina nimia* (Linton) was discovered in part. The metacercariæ are very commonly encysted in the thoracic region of *Lysmata intermedia* (Kingsley), a shrimp common among the *Porites* coral. The larvæ also occur, although less commonly, in the snapping shrimp, *Crangon formosum*. The adult trematode occurs in at least thirteen species of fishes. The fully developed metacercaria is recognized by the nine testes. The only other known *Helicometrina* (a new species) constantly has but five testes. Immature forms of *H. nimia* from fishes are indistinguishable from the metacercariæ from the shrimps. The cercaria of *H. nimia* is very possibly Cercaria J of Miller 1925 (Carnegie Inst. Wash. Year Book No. 24, p. 237) from *Columbella mercatoria*. At least, these cercariæ readily encyst in *Lysmata intermedia*. An overdose of the cercariæ proved fatal to four shrimps within a few hours. The cercariæ agree in all respects with *H. nimia* except in position of the pharynx.

Another partial life history became fairly apparent with the discovery that a Lepocreadid metacercarian very common as a free inhabitant (unencysted) in the intestine of many fishes of depths from 40 to 60 fathoms agreed in every respect with a species of *Lepidapedon* found in the intestine of *Epinephelus niveatus*. The very general occurrence, the sharply limited depth distribution, and the fact that the metacercarian is an unencysted, juvenile form make this life history of especial interest.

From the loggerhead turtle were secured several new records of trematodes of that host in America. These include the Aspidogastrid, *Lophotaspis vallei*. Eggs of this trematode were found to hatch readily. The miracidium is elongate-oval in shape, varying, according to contraction, from 150 to 210 μ in length. There is a large sucker at the posterior end. Two eye-spots, close together, occur dorsally about one-third from the anterior end. The long cilia are limited to three ciliated plates, one at the extreme posterior tip behind the posterior sucker, the other two lateral just behind mid-body. The plates appear as transparent low elevations, 20 μ in length. There is an elongate oral sucker at the anterior end, and an oval pharynx near the level of the eye-spots. No flame cells could be seen. The miracidium swims rapidly by means of its cilia, or it can creep into an inchworm, extending its anterior end, releasing its posterior sucker, then pulling the posterior end up near the mouth. Five different cases of abnormal hatchings were watched in which the miracidium was reversed in the egg shell so that the posterior sucker emerged first. Such hatchings were difficult and often abortive or incomplete.

Crustacea of the Tortugas Region, by Waldo L. Schmitt

The general survey of the crustacean fauna which has been carried on during the past several seasons, has just about been brought to a conclusion with the investigations conducted this year. The additional and

improved equipment provided on the *Anton Dohrn* rendered it possible to carry vertical observations down to nearly 600 fathoms—a depth far exceeding any haul yet attempted by the laboratory and a record for the *Dohrn*.

Of more than a dozen successful hauls this season from 100 fathoms or more, not less than six were made at depths between 290 and 580 fathoms. These have furnished extensive data bearing on a zone or zones not hitherto examined by us. It would seem that the bathymetric zonation of the deeper-water crustacea, foreshadowed last year, may be even more significantly indicated when the studies upon the materials secured will have been completed.

Noteworthy among the rarer forms obtained were several specimens of the Calappid crab, *Acanthocarpus bispinosus,* first described in 1880 by A. Milne Edwards from a single specimen taken by the *Blake* off the Grenadines in 140 fathoms, and so far as I am aware not again seen in the intervening half century. The Tortugas specimens are from 135 to 168 fathoms, south of Loggerhead Key. It may here be noted that this species and the only other known member of the genus, *A. alexandri* Stimpson, which has been so abundantly represented in the *Dohrn* dredgings between 60 and 110 fathoms, are both stridulating crabs. This fact seems to have passed unnoticed, though the vertical flattened file of finely cut, close set, oblique ridges on the inner face of the palm and the suborbital row of sloping, "dash"-like tubercles on the carapace against which they play are quite prominent structural features in these species. When touched or taken in the fingers under water, the crab may set up such a strong vibratory grating that the fingers are literally made to tingle.

Considerable new information regarding the food of Tortugas fishes was derived from the stomach contents of a number of species not examined in past years. Of particular interest among the crustacean records so established was the discovery of a small Pandalid shrimp in the stomach of a flounder, *Trichopsetta ventralis* Goode and Bean, from 70 fathoms. Though differing markedly in rostral armature, it is very close to *Parapandalus longicauda* (Rathbun), which has only been found in 88 fathoms in the upper Gulf of Mexico and off Porto Rico in 200 to 225 fathoms.

An additional specimen of *Pasiphæa merriami* described in last year's report and another large individual of the giant isopod, *Bathynomus giganteus,* a male approximately 13 inches in length, were secured this year.

The fair measure of success attending last year's attempts to record visually the life habits of some of the Tortugas reef dwellers encouraged a little more intensive work in this direction during the last days of the period I was enabled to spend in study at the laboratory. Results, so far as the development of the film has progressed, are much better than were those obtained the preceding season. Further studies were made of the burrowing jaw-fish, *Gnathypops,* constructing its home and showing resentment at the too near approach of other fish to the entrance of its retreat. In all, some 3200 feet of film were exposed.

Effects of Radium Radiations on Regeneration in Euratella chamberlin, by Raymond G. Stone

My studies on radiation and regeneration were continued from last summer. A more detailed investigation of the effects of combined beta and gamma radiations in comparison with those of the gamma rays alone

was undertaken. The sabellid, *Euratella chamberlin,*[1] used in these experiments has not previously been reported from this region although it is found in large numbers in the moat at Fort Jefferson.

Two groups of these worms were exposed simultaneously for varying periods in order to compare the differential effects of the rays. One group received the full output of beta and gamma rays from 123 mg. of radium. The other group was screened by a lead plate and received only gamma rays. The number and appearance of the new segments formed in posteriorly regenerating control-worms was the same as previously reported.[2] After removal of 4 to 6 anterior segments, normal worms regenerate only the head and collar segments at the cut surface. Histological examination of preserved material is necessary to discover whether the thoracic segments are replaced by transformation of abdominal segments as Berrill[3] reported in *Sabella pavonina.*

After 100 hours exposure to beta and gamma rays, the worms fail to replace anterior or posterior segments, though similar exposure to gamma rays alone has little effect upon regeneration. Apparently, therefore, the beta rays are chiefly responsible for the failure to replace lost segments. Sections of worms receiving the combined beta and gamma radiation show that wound healing is effected by rearrangement of adjacent old tissues without formation of new material. The new segments at the posterior ends of normal regenerating worms are mainly epithelial in origin. The epidermis and gut lining arise by proliferation of material from similar old tissues, while the nerve cord and mesodermal structures are formed from epidermal cells that push into the body cavity at the tip of the regenerating region. No undifferentiated reserve cells are found in *Euratella chamberlin,* although they are a source of new material during regeneration in other polychætes. The loss of regenerative powers after radiation in this worm is apparently due to direct effects of the rays within the epithelial cells.

Study of the Reef Corals of the Tortugas, by John W. Wells

The period of ten weeks, from June 1 to August 8, was spent at the Tortugas Laboratory. The study of the reef corals of the Tortugas was undertaken in connection with my work on fossil corals, a knowledge of the living corals and coral reefs being of great value in the interpretation of the more obscure fossil forms. The areal and bathymetric distribution of the different species, as well as the different growth-forms assumed under different conditions, was particularly emphasized. A total of 24 species (including two varieties or subspecies) of Madreporaria having a bathymetric range from low-water level to a depth of 25 feet was collected and examined. The distribution of the several types of associations of reef species was plotted on a chart of the Tortugas area.

During the dredging trips of the *Anton Dohrn,* specimens of eight species of "deep-sea corals" were taken in three hauls—at 39, 43 and 582 fathoms.

Experiments were conducted in an effort to determine the toleration of reef corals to increased salinity of sea-water. Specimens of the commoner reef species were placed in vessels containing sea-water evaporated to various concentrations, the salinity being roughly determined by titration.

[1] Identified by Dr. J. P. Moore, University of Pennsylvania.
[2] Carnegie Inst. Wash. Year Book No. 30, p. 395, 1930–31.
[3] Berrill, N. J., Jour. Exper. Zool., vol. 58, p. 495–523, 1931.

All of the specimens were placed in the water for 12-hour periods except in the case of salinity 50⁺, when the period was 6 hours. The results of these experiments are summarized in the following table:

Species	Salinity							
	40⁺	43⁺	46⁺	48±	50⁻	50⁺	55⁻	70⁻
Eusmilia fastigiata	Uninjured	.....	Uninjured	.....	Damaged	Recovered	Killed	.....
Oculina diffusa	.....	.....	.....	.....	.....	.....	Killed	.....
Orbicella annularis	Uninjured	Killed	Killed	Killed	Killed	Recovered	Killed	Killed
Orbicella cavernosa	.....	Uninjured	.....	.....	Do.	Do.	.....	.....
Favia fragum	Uninjured	Do.	Uninjured	.....	Uninjured	Uninjured	Injured	Killed
Mœandra areolata	Do.	Do.	Do.	Uninjured	Do.	Do.	Uninjured	Killed
Mœandra strigosa	Do.	Do.	.....	.....	Do.	Do.	Do.	Do.
Mœandra clivosa	Do.	Do.	Uninjured	Uninjured	Do.	Do.	Do.	Do.
Agaricia agaricites crassa	Do.	Damaged	Killed	.....	Killed	Recovered	Killed	.....
Siderastrea radians	Do.	Uninjured	Uninjured	Uninjured	Uninjured	Uninjured	Recovered	Killed
Siderastrea siderea	.....	Do.	.....	.....	Killed	Recovered	.....	.....
Porites porites	Uninjured	Do.	Killed	Killed	Killed	Killed	Killed	.....
Porites furcata	Do.	Do.	Do.	Do.	Do.	Do.	Do.	.....
Porites astreoides	Do.	Do.	Do.	Do.	Do.	Injured	.....	.....
Acropora muricata	Killed	.....	Killed	.....	.....	.....	.....	.....

These experiments, although of a preliminary nature, show that the hardier species of reef corals—*Favia fragum, Mœandra areolata, M. clivosa, M. strigosa* and *Siderastrea radians*—can endure a considerable increase in salinity at least for short periods of time, that most of the other species are killed by salinities in excess of 45, and that all are soon killed by a concentration greater than 55. It is probable, as Vaughan has inferred, that the reef corals of the Tortugas will not endure a concentration greater than the highest recorded for Tortugas water—36.29, for any considerable length of time.

Studies on the Nematodes of Tortugas Fishes, by O. L. Williams

More than 800 fishes representing about 175 species were examined for internal parasites during the summer of 1932. Although a careful study of the nematodes collected must be made before many conclusions can be drawn, a few preliminary observations may be recorded here.

A sufficient number of hosts has been examined to demonstrate rather clearly that the incidence of infestation with nematodes is somewhat lower in the fishes of the warm, shallow waters about the Tortugas than is the case in either the cooler, deeper waters of the same region or the cooler, shallow waters found farther north. Nematodes were collected from a

total of 80 species of fishes, or about 46 per cent of the different kinds of hosts examined. Inasmuch as the incidence of nematode infestation for a given species is usually low, further work will very materially increase the number of hosts known to bear nematodes occasionally.

Multiplication of species within a given host does not occur with the nematodes to the extent that it does with the trematodes. Not more than four kinds of nematodes were found in any one species of fish, and it is not expected that this number will be greatly increased. Furthermore, the number of nematodes parasitizing a single fish was found to be small in most cases. Extremely heavy infestations were found only in three groupers, *Epinephelus morio, Mycteroperca bonaci* and *Mycteroperca venenosa*. In these fishes, hundreds or even thousands of larval nematodes had become encysted throughout the viscera, being particularly abundant near the ends of the pyloric cœca.

Approximately two-thirds of the nematodes collected represent larval stages. Nearly all of these larvæ are sufficiently advanced, however, to make possible their identification. Although only a few of them have been determined as yet, it appears that many will prove to be the immature forms of species which normally mature in birds. With the completion of the study of the larval nematodes of fishes, a good starting point for life-history studies of the nematodes of marine birds may be found.